Mechanosensation: **Swimming round in circles**

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Studies of inherited deafness disorders in mice and humans are providing new insights into the basis of hair-cell mechanosensitivity; this enterprise has been joined by large-scale genetic screening in the zebrafish, where a number of intriguing mutants defective in mechanosensation have recently been described.

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Take a close look inside the ear — if you can get in there — and you will find an enormous variety of structure. The overall architecture of the inner ear is precise, individual cell types are clearly identifiable by shape and position, and there are proteins that are specific to the cochlea. The cochlea appears to be built in an orderly manner into a machine which can detect movements measured in nanometres and at rates measured in microseconds. The problem, of course, is that the workings of the inner ear are inaccessible and buried, at least within mammals, within a bony casing. And the amount of material, when you find it, is small.

Despite these aggravations, the orderly structure of the inner ear makes it a good arena for the application of molecular genetic techniques. During development, the vertebrate ear grows in a precise sequence of steps, and the ability to study the how and when of the specified genetic switches that are thrown would be a great attraction. There have recently been some remarkable successes in establishing which genes are involved in the development and function of the mammalian cochlea, progress that has often involved an interplay between mouse and human genetics. In turning to 'lower' vertebrates — birds, amphibia and fish — the developmental biologist finds that the mechanosensory structures can often be accessed and followed more easily. One particular system, the ear of the zebrafish, has advantages that have permitted a recent large-scale screen for mutations affecting development, and the fruits of this screen may now be on the verge of contributing to our understanding of mechanosensation.

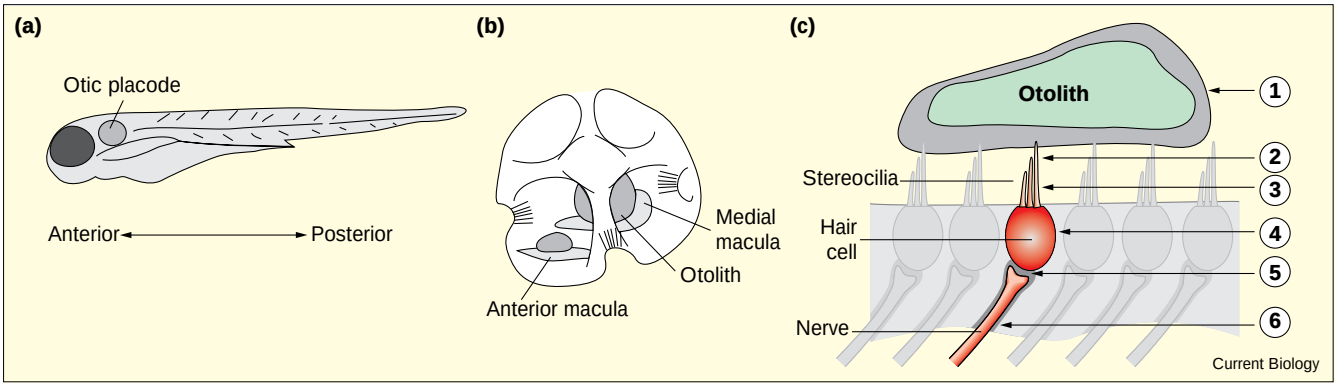
One of the first genes to be implicated in a specific deafness, the gene for myosin 7A [1,2], is associated with the mouse mutation *shaker-1* and the human mutation *Usher type 1B* (*DFNB2*). Although myosin 7A has now been shown to be localised to the sensory hair cells [3], it is not clear yet what its precise role might be. The preferred view is that this myosin is involved in membrane transport at the apical membrane [4] of the sensory hair cells. Myosin 7A is not the only 'unconventional' myosin in the cochlea, for myosin 6 has been associated with the deafness mutation *Snell's waltzer* in the mouse [5]; nothing is yet known about any possible cochlear function of the human homologue of this myosin, however. Other recent cloned genes implicated in cochlear function include the gene for the gap junction protein connexin 26, which is associated with the recessive deafness mutation *DFNB1* in humans [6], and the *HD1A1* gene, which is homologous to the *Drosophila* gene *diaphanous* and linked to the dominant mutation *DFNA1* in humans [7].

Although the number of genes linked to inherited deafness disorders is rapidly growing to a flood — five have been published in 1998 alone — some of these genes can affect other tissues as well as in the inner ear. Of particular interest, therefore, is the identification this year of a cochlea-specific gene which encodes the transcription factor POU4F3 (also known as BRN3.1 or BRN3c) and is associated with the human recessive deafness mutation *DFNB15* [8]. POU4F3 is involved in the early determination of tissue in the presumptive inner ear tissue to form hair cells. Mice lacking this gene fail to develop hair cells in the cochlea, and these knockout mice are deaf and have balance defects.

If a cell in the ear is to become a functional sensory hair cell — so-called because of the modified microvilli, or stereocilia, which project from the apical surface of the cell — it has to be able to convert mechanical deflections of its stereocilia into a neural signal. The transduction mechanism is sited at the ends of the stereocilia, and is believed to consist of channels which can be gated open by tension in a protein link ('tip link') generated when the stereocilia are deflected. This mechanism involves several ancillary proteins to tether the ion channel and provide tension to the link.

The cluster of transduction proteins in vertebrate hair cells has yet to be identified unequivocally, and the question of the identity of the hair-cell transducer remains an open one. There have been attempts to screen for proteins related to the 'degenerins' of the nematode *Caenorhabditis elegans*, but so far it is not clear which homologues are expressed and function in the cochlea. In *C. elegans*, the degenerin family includes two proteins, Mec4 and Mec10, which may form part of a mechanosensitive channel (reviewed in [9]). Other proteins in this family are likely to determine the linkages in the nematode cells

Figure 1



(a) The zebrafish ear. At five days, the embryo is about 3 mm long. (b) The otic placode, behind the eye, measures about 150 μm across at this stage; it contains hair cell epithelia as maculae and in the semicircular canals. (c) Sensing by hair cells requires integrity at multiple sites: 1, otoliths; 2, transducer complex in stereocilia; 3, stereocilia; 4, hair cell development; 5, synapse; and 6, auditory pathway. Genes in which mutations affect each site may have been identified in recent mutant screens.

that must be present before a mechanical stimulus can open the channel.

One promising approach is to use a vertebrate system that allows access to the mechanosensory structures and yet permits a rapid genetic analysis. The zebrafish fulfills these criteria, and it is a particularly promising model for studying inner ear development as many of the steps are enormously accelerated during formation of the zebrafish amniote. Although the inner ear of the zebrafish does not contain a cochlea for acoustic sound detection, it does possess, within the otic placodes behind each eye (Figure 1), organs for detecting the direction of gravity and semicircular canals for detecting dynamic rotation in three axes. Another major advantage of the zebrafish is that the embryo is quite transparent and the developing morphology of structures in the embryo can thus be followed.

The cavity for the otic placode becomes visible in zebrafish embryos at 18 hours, and by 42 hours hair cells are detectable in the sheets of cells that will become the maculae — gravity sensing epithelia — of the animal [10]. In five days of development, an inner ear structure has been formed, complete with the deposition of calcerous otoliths over the hair cell epithelium to detect vibration and to transmit motion to the hair cell stereocilia (Figure 1). In a large mutant screen carried out by Nusslein-Volhard and colleagues in Tübingen [11], 58 morphological mutant zebrafish were identified with a primary ear phenotype. The surprising result from analysis of these mutants is that many of the inner ear structures — the hair cells, otoliths and overall morphology, for example — can develop independently of each other. The number of mutations is comparable to the more than 50 identified mutations that affect hearing in the mouse.

In a more recent genetic analysis of the zebrafish inner ear, the strategy was to extend the screen to include mutants with abnormal swimming patterns [12]. These abnormal patterns include looping and rolling motions when the fish are disturbed or illuminated (which normally produces a righting orientation movement). In many of these these 'circler' mutants the inner ear structures appear structurally normal, although most are lethal because of the failure to develop a swim bladder or appropriate feeding behaviour. Eight groups of locomotion mutants have been identified — just enough not to exhaust the names of orbiting spacecraft after which they are named. The group of genes identified includes one, *skylab*, in which the mutant fish show clear signs of hair cell degeneration, suggesting a cell-survival factor may be involved.

The further interest in the work by Nicolson *et al.* [12] is that electrical measurements of inner-ear function were also made to augment their screen. Although hair cells are too small to record routinely with patch-clamp techniques, extracellular recording can elegantly measure the 'microphonic' current that flows through the transducer channels during deflection of the hair bundle in small groups of cells. These experiments can therefore decide whether the functional defect in a mutant is before or after the transducing step in the hair cell. There are two mutants, *sputnik* and *mariner*, where the hair bundle appears to be disorganised and mechano-electric transduction is compromised. Indeed, the microphonics are severely reduced, if not absent, in mutants caused by some alleles of these two genes. These mutant fish are reminiscent of myosin mutant mice [4] — severe myosin mutations can lead to progressive hair bundle disarray. The next step may be to look for myosin deficiencies in the zebrafish mutants.

Mutants in a second group, *orbiter*, *mercury* and *gemini*, show no hair cell microphonics at all, although their cell membrane physiology is reported as normal. This may be an indication that some member of the cluster of transduction proteins is the target in these mutants. Two mutants, *astronaut* and *cosmonaut*, have nearly normal hair cell microphonics, but transmission of neural signals from the hair cells to the midbrain regions is compromised, suggesting that the target of the mutations may be a component of the cell synapse or the neural pathway. There are as yet no mutations of the auditory pathway in mammalian systems that have been studied in depth at the single-cell level, so this may be a fertile area for further research.

This work reinforces the view that each sequential step in hearing and balancing may be determined by just a few genes, and each step must be intact in order for sensitivity to sound or movement cues to be apparent. This sensitivity to mutations in single genes has enabled some of the recent progress in unscrambling the mechanisms of deafness in mammals, where the link between molecule and behaviour is particularly striking. The wealth of data from studies of inherited deafness disorders in mice and humans has made gene identification possible, albeit on a slightly hit-and-miss basis. The introduction of the zebrafish model is an interesting new approach which may systematically open up questions about development and maintenance of the inner ear. The path is not completely straight, as the redundancy in the organisation of the zebrafish genome could make gene identification more difficult [13] and progress may be slower than one would like. Even so, the combination of genetic analysis and physiological dissection of the pathways is the obvious step forward.

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